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Relationship of Adipokines with Insulin Sensitivity in African

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Short Title: Adipokines and insulin sensitivity

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Abstract:

Cytokines produced by adipose tissue including adiponectin have been associated with metabolic abnormalities. **Objective:** The purpose of this study was to examine the relationship of insulin sensitivity measured by euglycemic hyperinsulinemic insulin clamp with plasma adiponectin and other adipokines in young adult African Americans. **Methods:** Participants were relatively healthy African Americans. Anthropometric measures, blood pressure, an oral glucose tolerance test and an euglygemic hyperinsulinemic insulin clamp were performed. Insulin sensitivity measurements were adjusted for percentage of fat mass. Plasma concentrations of adiponectin, plasminogen activator inhibitor 1 (PAI-1) and interleukin 6 (IL-6) were assayed on plasma from fasting blood samples. Pearson correlation coefficients and multiple regression models were fitted to assess the association between glucose sensitivity and cytokines. **Results:** In univariate analysis, there were statistically significant correlations of plasma adiponectin level (r = 0.19, P = 0.004), PAI-1 (r=-0.19, P=0.020) and IL-6 (r=-0.24, P<0.001) with measures of insulin sensitivity after adjustment for both fat mass and insulin clamp concentration. In multivariate analysis, adiponectin (GMR 1.15 P=0.007), PAI-1 (GMR 0.998, P=0.021) and BMI (GMR 0.95, P<0.001) were each independently associated with insulin sensitivity. For IL-6 there was no significant association with insulin sensitivity independent of obesity. **Conclusion:** This data show a significant and independent positive correlation of adiponectin with insulin sensitivity. The relationship of IL-6 with insulin sensitivity appears to be dependent on adiposity.

Key Words: adipokines, adiposity, insulin resistance.

Introduction:

Adipose tissue is recognized not only as an energy reserve organ but also as an endocrine organ as it produces several cytokines and hormones designated as "adipokines".[1,2] Cytokine involved in inflammation that are secreted in adipose tissue include plasminogen activator inhibitor type 1 (PAI-1), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), leptin, and resistin. Adipocytes also produce adiponectin, a hormone that has been associated with anti-inflammatory effects. There appears to be a close association between inflammation and obesity given the increased levels of pro-inflammatory cytokines and decreased level of adiponectin observed in obese individuals compared with non-obese individuals.[3-5] Obesity related inflammation has been proposed as a possible mechanism by which obesity increases insulin resistance and leads to diabetes.[6-8] Some data suggest that the condition of chronic subclinical inflammation can also induce endothelial dysfunction in the vascular bed and hypercoagulability, which facilitate vascular injury leading to cardiovascular disease.[9] The extent to which adipokines induce metabolic abnormalities in humans is not fully resolved.[9,10]

Adiponectin is the most abundant adipokine and is synthesized exclusively in adipose tissue.[11] Animal studies have shown that adiponectin plays an important role in regulating insulin sensitivity by inhibiting hepatic gluconeogenesis and stimulating fatty acid oxidation in liver and skeletal muscle by activating AMP kinase[12] and peroxisome proliferator–activated receptor alpha.[13-15] In addition, adiponectin deficient mice have been shown to be insulin resistant[16] and administration of exogenous adiponectin improved insulin sensitivity.[14] Because insulin resistance is considered a pre-diabetic state, the insulin sensitizing effects of adiponectin suggests an anti-diabetic biologic role.

African Americans have lower adiponectin levels than Caucasians, Hispanics and Asians independently of obesity.[17,18] In addition to lower plasma adiponectin levels, African Americans present similar or higher expression levels of inflammatory markers such as CD68, IL-6 and TNF-α in the adipose tissue.[19] This unfavorable adipokine profile may underlie the heightened risk for diabetes in African Americans.

The purpose of this study was to examine the relationship of insulin resistance with adiponectin and other adipokines in young adult African Americans. We hypothesized that insulin resistance in African Americans is associated with lower adiponectin level and higher inflammatory cytokines and that the association of cytokines with insulin sensitivity is independent of body fat mass.

Methods:

Study Population

The study was conducted on African Americans living in urban Philadelphia aged 28-52 years. Participants in this study were drawn from a cohort of healthy young adult African Americans enrolled in a longitudinal study of blood pressure and risks for cardiovascular and renal injury who were previously examined between 1994 and 1999. Participants were re-enrolled for this project between August 2001 and July 2007.

Exclusion criteria for enrollment in 1994-1999 included secondary forms of hypertension, diabetes type I or type II, renal or cardiac disease, autoimmune disease and polycystic ovary syndrome. Individuals who subsequently developed type II diabetes, as well as participants found to be diabetic on re-examination, were included in the study. The study protocol was approved by the Institutional Review Board of Thomas Jefferson

University. Written informed consent was obtained from each participant at re-enrollment.

Study Procedures

Each participant was examined on two separate visits, 4-8 weeks apart. Clinical assessment at both visits consisted of anthropometric measurements (height, weight, waist circumference, and skinfold measurements), and blood pressure (BP). Data on health status and health related behaviors were obtained by participant self-report. An oral glucose tolerance test (OGTT) was performed on the first visit and a euglycemic hyperinsulinemic clamp was performed on the second visit

Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). BP measurements were obtained on each subject following a 10-minute rest period in a seated position using auscultation with a mercury column sphygmomanometer. The average of three successive readings of systolic and diastolic pressure was used as the BP values of each visit.

An OGTT was conducted after a 12-hour overnight fast and a fasting blood sample was also obtained for plasma glucose and insulin. A residual aliquot of plasma from the fasting blood sample on each participant was stored at -80º C. Following the ingestion of 75 g of glucose solution (Glucola; Ames Diagnostics, Elkhart, IN), blood samples were obtained at 30, 60 and 120 minutes post-ingestion and assayed for plasma glucose and insulin concentrations. Plasma glucose concentration was analyzed with the glucose oxidase technique (YS Model 27; Glucostat, Yellow Springs, OH). Plasma insulin concentration was determined with a solid phase radioimmunoassay (RIA)(Coat-a-Count; Diagnostic Products Corp, Los Angeles, CA). Coefficients of variation for

intra- and inter-assay variability for glucose and insulin assays were <5%. A blood sample for fasting lipids was obtained during the first visit. Total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides were measured using the Hitachi 704 standard enzymatic method in the Lipid Laboratory of Thomas Jefferson University.

The euglycemic hyperinsulinemic clamp was carried out as previously described.[20-22] In brief, all subjects were required to have a 12-hour overnight fast before the insulin clamp procedure. Blood samples were obtained for baseline plasma glucose and insulin concentration. Hyperinsulinemia was established with a primed constant infusion of insulin (Eli Lilly, Indianapolis, IN) at a concentration of 1000 mU/ml in normal saline according to the method of Rizza et al.[22] The primed infusion rate was sufficient to achieve steady-state hyperinsulinemia at 80-120 μU/ml above fasting insulin levels with the goal of suppressing hepatic glucose production. Hyperinsulinemia was maintained for 120 minutes, during which time plasma glucose concentration was maintained at fasting (baseline) level using a variable infusion of 20% dextrose in water (Abbott Lab, Abbott Park, IL). For participants with elevated fasting glucose greater than 110 mg/dl, baseline glucose was set at 100 mg/dl as target euglycemic level. The glucose infusion rate was adjusted by the negative feedback equation of DeFronzo et al[21] according to plasma glucose sampled every 10 minutes. The calculated mean value for the glucose infusion rate (M in mg/kg per min) during the final 60 minutes of the clamp procedure was the measure of insulin-stimulated glucose uptake, or insulin sensitivity. Using anthropometric measures, we computed the fat-free mass for each subject using the Durnin and Womersley formula,[23] and the insulin-stimulated glucose uptake was

expressed as M' (mg/Kg of fat-free mass per min). M' was adjusted for the level of steady-state hyperinsulinemia in each case by dividing M' by the mean plasma insulin concentration (I in µU per ml) during the final 60 minutes of the clamp procedure to derive an insulin sensitivity index (M'/I in mg/kg fat-free mass per min/µU per ml x100).

 Fasting plasma samples that had been stored at -80ºC were used to assay a panel of cytokines considered to be biomarkers of inflammation including IL-6, PAI-1, and the anti-inflammatory cytokine adiponectin. All assays for the cytokines were performed by ELISA in duplicate using commercially available kits. Kits for Adiponectin, and IL-6 were obtained from R&D Systems (Minneapolis, MN). The kits for PAI-1 were obtained from Aniara (Mason, OH). The coefficient of variation for these assays was consistently <10% and most <6%.

Statistical Analysis:

Continuous variables considered for analysis of insulin sensitivity included age, BMI, adiponectin, IL-6, and PAI-1. Categorical variables included gender and smoking status (response to "do you smoke cigarettes? yes or no"). Due to the skewed distribution of the M'/I x100 data, M'/I x100 was natural log transformed for statistical analysis as a continuous dependent variable. The data for M/I x100, adiponectin, and IL-6 also had skewed distributions and were log transformed for analysis. For each of these variables, the geometric mean and the first and third quartiles on the original scale were presented as measures of central tendency and dispersion.

To summarize tabulated data, subjects were stratified as either having normal or abnormal glucose tolerance based on their fasting plasma glucose and OGTT results according to ADA criteria:[24] normal glucose tolerance was defined as fasting plasma

glucose < 100 mg/dL and two hour post OGTT glucose < 140 mg/dL and abnormal glucose tolerance was defined as fasting plasma glucose ≥ 100 mg/dL or two hour glucose ≥ 140 mg/dL. BMI status was classified as normal weight (BMI < 25 kg/m^2), overweight (25 ≤ BMI <30 kg/m^2), or obese (BMI ≥ 30 kg/m^2). T-tests (assuming unequal variances) were used to determine if the means of risk factors or cytokines were different between the glucose tolerance groups and Fisher's exact test was used to test for independence between categorical variables and abnormal glucose tolerance.

To investigate unadjusted linear relationships between continuous M, M' or M'/I x100 and adipose tissue cytokines, we calculated and tested the significance of Pearson correlation coefficients. Associations between insulin sensitivity (M'/I x100) and cytokines adjusted for other study variables were evaluated by multiple linear regression models on log M'/I x100 as a dependent variable. Model parameters for independent variables from these models are presented as geometric mean ratios (GMR) which individually represented the independent multiplicative increase in M'/I x100, on its untransformed scale, per unit increase of a continuous independent variable (i.e., age, BMI, adiponectin, IL-6, or PAI-1) or per categorical indication (i.e., female or smoker). There were 80 subjects (34%) missing PAI-1 measurements and thus we analyzed the adjusted association between log M'/I x100 and PAI-1 separately from adiponectin and IL-6.

Results

The study cohort consisted of 235 participants, who were stratified as normal glucose tolerance (NGT, N = 158) and abnormal glucose tolerance (AGT, N = 77). **Table 1** provides the clinical characteristics of study participants. Mean age of the participants was 40 years and there was no significant age difference between the NGT and AGT groups. In the total study sample, 64% were females and 44% were self-identified cigarette smokers with no differences by glucose tolerance groups. Mean BMI values for both NGT and AGT groups were in the obese range (BMI $\geq$30 kg/m^2). However, the AGT group had a significantly higher mean BMI value than the NGT group. Normal BMI (<25 kg/m^2) was present in 20% of the NGT group compared to 10% in the AGT group, but this difference did not reach statistical significance (p=0.054). There were no differences in lipid parameters between glucose tolerance groups. As expected, fasting glucose and fasting insulin were significantly higher in the AGT group (p<0.001 for both). Mean systolic and diastolic BP values were not significantly different between NGT and AGT groups.

Table 2 provides data on insulin sensitivity, derived from the insulin clamp procedure and cytokine data for the two glucose tolerance groups. Mean glucose infusion rate (M) and insulin sensitivity index (M/I x100) with and without adjustment for total body fat were significantly lower, indicating greater insulin resistance in the AGT group, (p<0.001 for all comparisons). Adiponectin levels were lower in the AGT group but the difference was not statistically significant. Il-6 and PAI-1 levels were significantly higher in the AGT group compared to the NGT group (p=0.041 and 0.048 respectively).

Correlation coefficients between the clamp measures of insulin sensitivity with each of the cytokine parameters in the entire cohort are presented in **Table 3.** Although the mean adiponectin concentration was not significantly different between glucose tolerance groups, there were statistically significant correlations of plasma adiponectin level with measures of insulin sensitivity (M), r=0.22, P< 0.001. When M was corrected for adiposity and expressed as mg/Kg of fat free mass (M') the correlation coefficient was 0.25 (P<0.001). The correlation coefficient remained significant (r = 0.19, P = 0.004) when corrected for clamp insulin concentration (M'/Ix100). There was a significant negative correlation of PAI-1 with insulin sensitivity that remained significant following correction for clamp insulin concentration (r=-0.19, P=0.020). The correlation of this cytokine with insulin sensitivity measures changed minimally after adjustment for body fat mass. There was also a significant correlation of IL-6 with insulin sensitivity that remained significant after adjusting for clamp insulin concentration and body fat mass (r=-0.24, P<0.001).

We then performed multiple regression analyses to determine if there were independent correlations of adiponectin, IL-6 and PAI-1 with insulin sensitivity expressed as M'/I x100. In these regression models, smoking status, gender, age, and BMI were also included as independent variables. **Table 4** provides results of the regression analysis of adiponectin and IL-6 with results presented as geometric mean ratios. In this model there was a significant independent effect of BMI on insulin sensitivity (GMR 0.95, P<0.001). In addition there was also a significant independent effect of adiponectin on insulin sensitivity (GMR 1.15 P=0.007). No significant independent effect was detected for IL-6 (GMR 1.02, P=0.769) or the other variables in

this model. Similar analyses were performed to examine PAI-1. For PAI-1, there was a significant independent effect for PAI-1 on insulin sensitivity (GMR 0.998, P=0.021), despite the reduction in number of subjects with PAI-1 values (N = 155 cases, data not shown). These results indicate that adiponectin correlates with insulin sensitivity independent of BMI, and that the correlation of IL-6 with insulin sensitivity is dependent on or at least highly collinear with BMI.

Discussion

Our data on a young adult African American cohort demonstrate a significant

positive relationship of plasma adiponectin level with insulin sensitivity quantified by the

insulin clamp procedure. Lower insulin sensitivity, or insulin resistance, was associated

with lower plasma adiponectin level. This relationship remained statistically significant

when insulin sensitivity measures were corrected for body fat. Additional regression

models provide further evidence that the relationship of adiponectin with insulin

sensitivity is independent of BMI. PAI-1 was also found to have a modest but significant

negative relationship with insulin sensitivity. Although a significant negative correlation

of IL-6 with insulin sensitivity was found in unadjusted analyses, multiple regression

analysis detected no significant independent association of IL-6 with insulin sensitivity,

indicating that the relationship of IL-6 with insulin sensitivity is dependent on obesity.

Adiponectin is thought to have anti-inflammatory and antidiabetic properties in

humans with recent studies reporting an inverse relationship between plasma adiponectin

level and insulin resistance,[16,25] metabolic syndrome,[26] diabetes,[17,27] and cardiovascular

disease.[16,28] There is also a consistently observed inverse relationship between

adiponectin and obesity. Weyer et al[29] investigated the relationship of adiponectin with

insulin clamp measures of insulin sensitivity in Pima Indians and reported that the low

plasma adiponectin concentration was more directly related to with insulin resistance

than to adiposity or glucose intolerance. These observations in Pima Indians were

extended by Stefan et al[30] who reported, in a prospective study, that low plasma

adiponectin levels precede a decline in insulin sensitivity. Subsequent investigations in

Caucasians also found a significant positive relationship of adiponectin with insulin

sensitivity that was independent of body fat.[31] In another study, Abbasi et al,[32] stratified a modest sample of both obese and non-obese subjects according to insulin sensitivity or insulin resistance and compared their plasma adiponectin levels. These investigators found higher plasma adiponectin levels among insulin sensitive subjects compared to those with insulin resistance, suggesting that the primary association of adiponectin was with insulin sensitivity rather than obesity. Our data in this study advance these findings to African Americans and demonstrate an independent association of adiponectin with insulin sensitivity in terms of insulin mediated glucose uptake.

Previous reports have described a relationship of low plasma adiponectin and insulin resistance in African Americans.[33-35] However, these clinical studies have relied on calculated estimates of insulin sensitivity such as homeostasis model assessment, based on fasting glucose and insulin concentration. The hyperinsulinemic euglycemic insulin clamp is a standard procedure to quantify insulin sensitivity in humans.[36, 37] Although the procedure is technically difficult, it represents a direct measure of insulin sensitivity by whole body insulin stimulated glucose uptake.[38] Our study represents one of the largest African American cohort, in which the insulin clamp procedure was used to investigate the relationships between adipose-derived cytokines and insulin sensitivity.

Several studies have investigated the relationship of inflammation and insulin resistance. Hotamisligil et al measured TNF-α protein in subcutaneous adipocytes from obese and non-obese women. Compared to non-obese women, obese women had higher adipocyte TNF-α and higher plasma insulin, an indirect measure of insulin resistance.[7] Subsequently, several investigators have reported an inverse association between insulin sensitivity and cytokines involved in inflammation such as C-reactive protein (CRP),

TNF-α and PAI-1[8,39,40] with some evidence that the cytokine effect was independent of BMI. Pscherer et al[41] demonstrated improvement in insulin resistance in obese men with impaired glucose tolerance following treatment with valsartan. These investigators also demonstrated a parallel decrease in plasma CRP and increase plasma adiponectin following treatment with the renin angiotensin system blocker, suggesting an anti-inflammatory effect of treatment on insulin resistance. Most reports on the relationship of insulin sensitivity with IL-6 have been based on changes in insulin sensitivity and IL-6 following weight loss.[42,43] Vozarova et al[44] studied the relationship of IL-6 with insulin sensitivity measured by euglycemic insulin clamp in Pima Indians without diabetes. Plasma IL-6 levels correlated negatively with insulin sensitivity but the correlation did not remained significant once the insulin mediated glucose uptake (M) was adjusted for body fat. The results of our study are consistent with these findings which demonstrate that the relationship of IL-6 with insulin sensitivity is mediated through fat mass.

Our study has some limitations. The results are based on a cross-sectional design study and significant correlations do not demonstrate causality. Our study used BMI as a surrogate of body adiposity. We did not distinguish body fat distribution or quantify visceral fat mass. It is known that BMI is a measure of excess weight rather than excess fat and does not differentiate between increase in fat, muscle or skeletal weight. Because of this BMI can be misleading as a body fat indicator especially in the extremes of age (children and older people). There is also a racial disparity with African American women having lower fat mass than Caucasian women for a same given BMI. [45] Although BMI does not reflect body fat distribution, results of previous studies indicate that different patterns of fat accumulation do not fully explain the excess prevalence of

diabetes among African Americans.[46] On the other hand BMI seems to be fairly accurate as a predictor of body fat within sex-age groups and distinguish categories of percentage body fat.[47]

Plasma adiponectin levels in this study seem to be somewhat higher than adiponectin levels measured in more recent studies in our laboratory on African American participants.[48] Since the samples used in this study were stored for several years there is a possibility that some evaporation of the samples could have occurred. If that was the case, we assume the evaporation happened uniformly and even though the adiponectin levels are higher than expected the overall results and associations are still valid.

In summary, our data demonstrate that adiponectin is significantly and positively associated insulin-stimulated glucose uptake, independent of fat mass. There is a modest negative relationship of the adipokine PAI-1 with insulin sensitivity that is independent of fat mass. We also demonstrated that the relationship of IL-6 with insulin sensitivity is lost following adjustments for BMI indicating that the relationship of IL-6 with insulin sensitivity is dependent on adiposity. Further studies are needed to clarify the mechanism by which adipokines affect human metabolism independent of obesity.

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Tables 1. Clinical Parameters per Glucose Tolerance Group

Variable	All Subjects (N = 235)	Normal GT (N = 158)	Abnormal GT (N = 77)	p-value
Gender Female	151 (64%)	98 (62%)	53 (69%)	0.384[*]
BMI Group Normal	40 (17%)	32 (20%)	8 (10%)	0.054[*]
Overweight	67 (29%)	48 (30%)	19 (25%)	
Obese	128 (54%)	78 (49%)	50 (65%)	
Age (yrs)	40.0 (3.5)	39.9 (3.5)	40.1 (3.7)	0.636[†]
BMI (kg/m^2)	31.8 (7.7)	30.9 (7.5)	33.8 (7.8)	0.008[†]
BP Systolic (mmHg)	125.9 (19.3)	124.9 (20.3)	128.1 (16.9)	0.193[†]
BP Diastolic (mmHg)	73.4 (12.6)	73.6 (13.6)	73.1 (10.5)	0.768[†]
Fasting Glucose (mg/dl)	102.3 (22.3)	97.6 (8.1)	112.1 (35.3)	<.001[†]
Fasting Insulin (µU/ml)	10.5 (8.7)	8.8 (6.2)	14.1 (11.5)	<.001[†]
HDL-cholesterol (mg/dl)	48.6 (13.3)	49.3 (14.0)	47.2 (11.7)	0.236[†]
LDL-cholesterol (mg/dl)	118.2 (33.7)	118.9 (35.5)	116.8 (30.0)	0.654[†]
Triglycerides (mg/dl)	87.5 (56.1)	83.6 (55.8)	95.1 (56.4)	0.144[†]
Total Cholesterol (mg/dl)	182.5 (36.2)	182.9 (37.2)	181.9 (34.5)	0.848[†]

Categorical variables are presented as frequency (%) and continuous variables as mean (SD)

GT: Glucose Tolerance, BMI: Body mass index, BP: Blood Pressure

[*] Fisher's exact test

[†] t-test

23

Table 2. Insulin Sensitivity Measures and Cytokine Values by Glucose Tolerance Group

Variable	All Subjects (N = 235)	Normal GT (N = 158)	Abnormal GT (N = 77)	p-value (t-test)
M (mg/kg per min)[*]	5.7 (2.8)	6.4 (2.9)	4.3 (2.1)	<.001
M' (mg/kg FFM per min)[*]	8.9 (4.1)	9.9 (4.2)	6.9 (2.9)	<.001
M/I x100[†] (mg/Kg per min/µU per mlx100)	6.8 [4.2, 12.2]	8.3 [5.3, 13.1]	4.6 [3.0, 7.9]	<.001
M'/I x100[†] (mg/Kg FFM per min/µU per mlx100)	10.9 [6.9, 18.0]	13.0 [8.9, 20.3]	7.6 [5.3, 14.2]	<.001
Adiponectin (µg/ml)[†]	9.1 [5.8, 14.6]	9.5 [6.0, 14.6]	8.3 [4.9, 14.2]	0.171
Interleukin 6 (pg/ml)[†]	2.5 [1.6, 3.6]	2.4 [1.6, 3.4]	2.9 [1.7, 4.0]	0.041
PAI1 (ng/ml)[* ‡]	138.3 (72.9)	129.7 (68.4)	155.5 (79.0)	0.048

[*] Arithmetic means with (SD) presented

[†] Data natural log transformed: geometric means with [1st quartile, 3rd quartile] presented

[‡] N = 155

GT: Glucose Tolerance

FFM: Fat Free Mass

I: steady-state insulin concentration during clamp in µU per mL

Table 3. Correlations Coefficients (r) Between Cytokines and Insulin Sensitivity

Measurements

Insulin Sensitivity	r (p-value) Adiponectin*	Interleukin-6*	PAI1
M (mg/Kg per min)	0.22	-0.23	-0.21
	(<.001)	(<.001)	(0.008)
M' (mg/Kg FFM per min)	0.25	-0.17	-0.22
	(<.001)	(0.008)	(0.005)
M'/I x100 * (mg/Kg FFM per min)/µU per mlx100	0.19	-0.24	-0.19
	(0.004)	(<.001)	(0.020)

*Data natural log transformed

FFM: Fat Free Mass

I: steady-state insulin concentration during clamp in µU per mL

Tables 4. Multiple Regression Model for Insulin Sensitivity

(M'/I x100) [*]

	Geometric Mean Ratio	(95% CL)	p-value
Age (yr)	0.997	(0.976, 1.019)	0.804
BMI (unit)	0.95	(0.93, 0.96)	<.001
Gender (Female)	1.15	(0.98, 1.36)	0.078
Smoking	1.09	(0.94, 1.27)	0.250
Adiponectin [*]	1.15	(1.04, 1.27)	0.007
Interleukin-6 [*]	1.02	(0.89, 1.17)	0.769

[*] Data natural log transformed